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Layered Nanoarchitectonics with Layer-by-Layer Assembly Strategy for Biomedical Applications

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Rapid progress in nanotechnology has greatly stimulated the development of nanomaterials with advanced functions. A large variety of nanomaterials including nanoparticles, nanotubes, nanosheets, and nanoporous materials have been fabricated. However, their individual functions are limited. Architecting nanomaterials into organized structures is an interesting way to construct advanced functional materials. Thus, a new concept for construction of functional materials from nanoscale building units is required. This is *nanoarchitectonics*, introduced firstly by Prof. Aono in 2000. Briefly, it is a paradigm about ordering building blocks at the molecular and macromolecular levels to achieve specific functions and cooperative properties [1, 2]. The concept is now widely accepted by materials preparation, structure fabrication, and various applications including catalysis, energy technology, environment, and biomedicine [3]. According to nanoarchitectonic strategies, materials are formed on the basis of manipulation of atoms, molecules, or their assemblies through synergetic combinations of chemical nanofabrication, self-organization, and field-controlled organization [4]. Now, a large variety of nanoarchitectures constructed with these strategies have been presented, including layered architecture. The layered architectures could be excellent prototypical systems for construction of 3D nanoarchitectures since forming materials precisely from atoms or molecules into complex 3D systems remains a difficult matter.

In film nanoarchitectonics, three major strategies are used for thin-film preparation including self-assembled monolayer (SAM) technique [5, 6], Langmuir–Blodgett (LB) method [7, 8], and layer-by-layer (LbL) assembly technique. Among these methods, the LbL assembly technique, which is most simple and versatile, has unlimited applicability to incorporate a wide range of building blocks into the layered architectures and has the ability to functionalize a large variety of substrates of any shape and any dimension. The approach can be integrated into the nanoarchitectonics concept to obtain versatile layers with precise and predictable architecture.

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7.1 Layer-by-Layer Assembly Technique

7.1.1 Basics of LbL

The LbL assembly technique, firstly proposed by Decher and coworkers in 1991, is based on the alternating deposition of oppositely charged polyelectrolytes onto a charged substrate [9–12], whose scheme is shown in Figure 7.1. All the deposition processes are performed under mild aqueous conditions with low-cost manufacturing. It is not limited to the planar substrates and is applicable to spherical, porous, and nonporous substrates [13–16]. At the beginning, the driving force for the deposition was the electrostatic interactions between the polyelectrolytes. Nowadays, various types of chemical or biological interactions such as hydrogen-bond, covalent interaction, coordination, and DNA hybridization have been also utilized to construct multilayers [17–20]. Correspondingly, a large variety of materials including lipids, DNA/RNA, proteins, inorganic nanosystems, and organic dyes could be used as building blocks for multilayer construction [21–26].

Now there are three main methods for applying the LbL coating to a surface. They are dipping coating, spin coating and spray coating.

7.1.2 Dipping Coating

Dipping coating is the most commonly used method for the LbL assembly. It is not restricted to any specialized equipment. Typically, the materials to be deposited are stored as a reservoir. The substrate will be exposed to and immersed into the reservoirs alternately in an appropriate order. The process is repeated until the desired number of layers is achieved. Dipping coating is simple, but it is time consuming due to the time period for equilibrium absorption at each deposition step. Thus, a combination of the LbL coating with other techniques is required to address the problem of long coating time. Spin-coating LbL assembly is such a strategy combining spin coating with the LbL assembly for thin-film preparation.

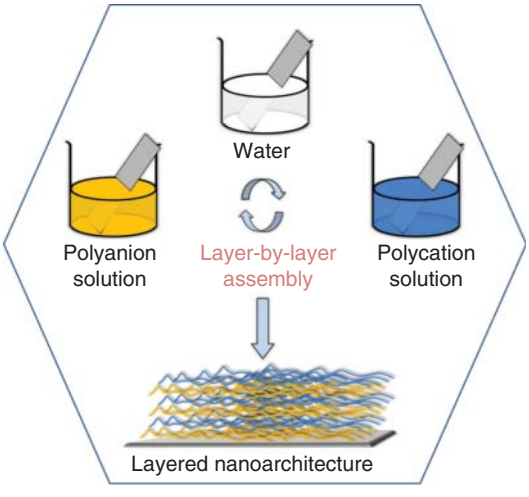

Figure 7.1 Schematic representation of the formation of layered nanoarchitectures with layer-by-layer assembly technique.

7.1.3 Spin Coating

Spin coating is a technique used for casting chemical layers onto a rotating substrate [27]. Exactly, a liquid is deposited and spread across a planar surface through rapid spinning of the substrate, as shown in Figure 7.2a. The process is rapid and significantly reduces the time for film preparation. For example, in 2001, Char and coworkers reported a spin-coating LbL assembly method as an alternative for making well-organized multilayer films in a very short process time (Figure 7.2b) [28]. Subsequently, Johal *et al*. investigated the application of the method to a variety of polyelectrolytes including polymers and dendrimers [29]. In addition, the method could be viable for producing a

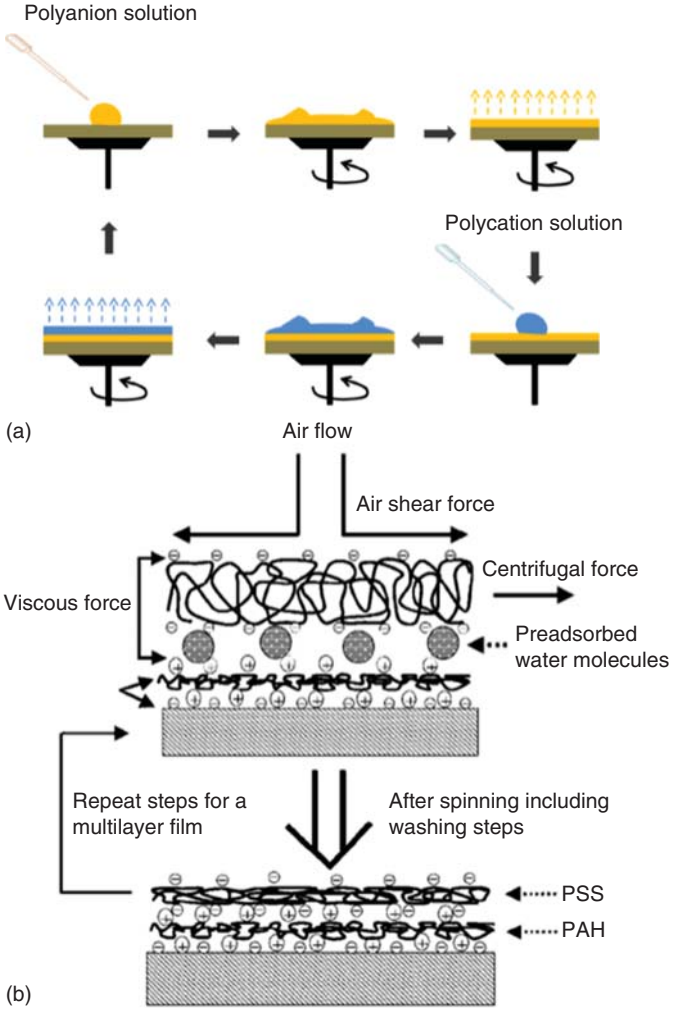

Figure 7.2 (a) The schematic representation of layer-by-layer spin coating process of polyelectrolytes. (b) A side view schematic depicting the build-up of multilayer assemblies by consecutive spinning process of anionic and cationic polyelectrolytes. (Cho *et al*. 2001 [28]. Reproduced with permission of John Wiley & Sons.)

structured nanocomposite by incorporating nanocrystalline cellulose within polymer matrix [30]. In contrast with the conventional dipping process, the spin-coating LbL assembly process has been reported to yield thick layers with increased concentration of polymers and highly ordered internal structure due to the centrifugation force and air shear force caused by the spinning process [31]. In fact, the primary advantage of the spin-coating LbL assembly over the dipping coating is the ability to tune thickness, density, roughness, and other properties by changing parameters such as system concentration, system viscosity, solvent type, and spin speed [32, 33]. However, the applicability of this method to the fabrication of layers on irregular curved structures and three-dimensional substrates remains a challenge.

7.1.4 Spray Coating

Spray coating is a method with advantages over the dipping and spin coating. For instance, the dipping process requires a deposition time of 15–20 min per layer to reach equilibrium, while for the spray coating 6 s for one layer is enough. Also, unlike spin coating, spray-coating LbL assembly enables homogeneous coating of 3D substrates. It has been reported that the time-efficiency assembly process of the spray coating leads to films with small surface roughness [34]. Importantly, Hammond *et al*. have made an attempt to develop an automated apparatus capable of spray depositing LbL [35], which lays the groundwork for scale-up construction of layer architectures capable of coating much larger substrates as well as 3D substrates. This will make the LbL assembly technology attractive on an industrial scale.

7.2 LbL-Assembled Layer Architectures with Tunable Properties

The most characteristic feature of the LbL assembly technique is the ability to tune the properties of the assembled layers by varying some parameters like pH, ionic strength, number of layers, and concentration of building block [36–39]. For example, we have investigated that the swelling property of the polyelectrolyte multilayers (PEMs) containing poly(diallyldimethylammonium chloride) (PDDA) and poly(sodium 4-styrenesulfonate) (PSS) in cell culture media could be tuned by changing supporting salt solutions during the assembly process (Figure 7.3) [40]. Specifically, the films assembled in low salt concentration (i.e., 0.15 M NaCl) or no salt poorly swelled in cell culture media, while for those assembled in higher salt concentration like 0.3, 0.5, and 1.0 M NaCl, they swelled significantly to a maximum of 400%. The swelling properties have been found to have an effect on cell adhesion onto the films, which provided an efficient strategy to fabricate the desired and engineered cell-interacting materials.

In another system shown in Figure 7.4, the LbL-assembled graphene oxide (GO) nanocomposite films were constructed, aimed at improving the mechanical properties of native polymer films [41]. It was demonstrated that the elastic modulus (E_r) could be improved by 181% when there was one

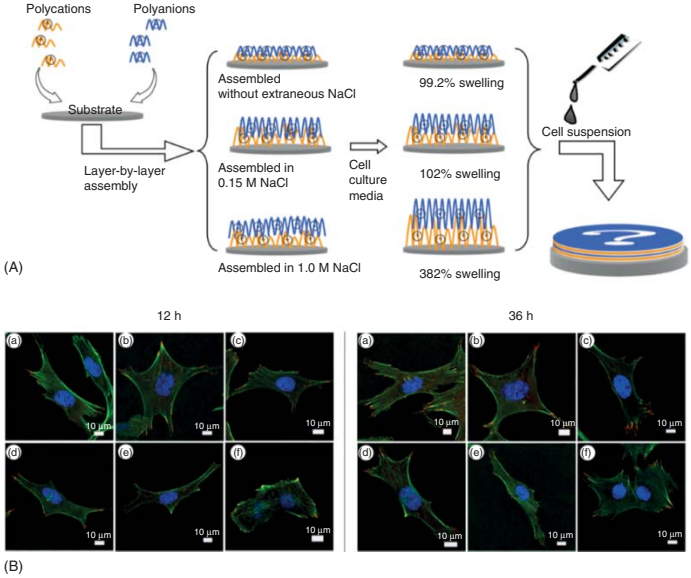

Figure 7.3 (A) Schematic representation of the effect of swelling of the PDDA//PSS multilayer films assembled in different NaCl solutions on NIH-3T3 cell adhesions. PDDA, poly(diallyldimethylammonium chloride); PSS, poly(sodium 4-styrenesulfonate). (B) Fibroblast morphology and cell adhesions on the PEI/PSS/$(PDDA/PSS)_9$ multilayer films assembled in different NaCl solutions after being plated for 12 h and 36 h, respectively: (a) without extraneous NaCl; (b) 0.15 M NaCl; (c) 0.3 M NaCl; (d) 0.5 M NaCl; (e) 1.0 M NaCl, and (f) on bare glass coverslip. For NIH-3T3 cells, actin and vinculin were stained with Fluorescein isothiocyanate (FITC)-phalloidin (bright gray) and a monoclonal anti-vinculin antibody (light gray), respectively. (Qi *et al*. 2014 [40]. Reproduced with permission of John Wiley & Sons.)

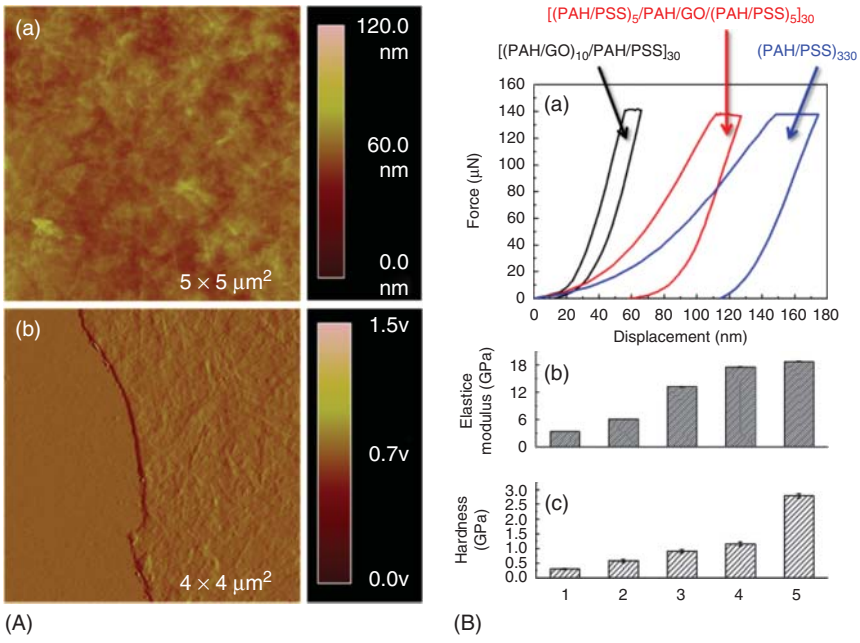

Figure 7.4 (A) AFM images showing surface morphology (a) and the phase images (b) of $(PAH/GO)_{10}/PAH/PSS$ multilayer films. (B) (a) Force–displacement curves of $(PAH/PSS)_{330}$, $[(PAH/PSS)_5/PAH/GO/(PAH/PSS)_5]_{30}$, and $[(PAH/GO)_{10}/PAH/PSS]_{30}$ films. Values of elastic modulus (b) and hardness (c) for the LbL multilayer films: 1, $(PAH/PSS)_{330}$; 2, $[(PAH/PSS)_5/PAH/GO/(PAH/PSS)_5]_{30}$; 3, $[(PAH/PSS)_2/PAH/GO/(PAH/PSS)_2]_{66}$; 4, $(PAH/PSS/PAH/GO/PAH/PSS)_{110}$; 5, $[(PAH/GO)_{10}/PAH/PSS]_{30}$. (Qi *et al*. 2014 [41]. Reproduced with permission of Royal Society of Chemistry.)

layer of GO deposition in 10 bilayers of polyelectrolyte, while the E_r value of $[(PAH/GO)_{10}/PAH/PSS]_{30}$ film showed more than fivefold enhancement over the native PEM film $(PAH/PSS)_{330}$. The hardness (H) also increased significantly, from 0.295 to 2.79 GPa for the $(PAH/PSS)_{330}$ film and the $[(PAH/GO)_{10}/PAH/PSS]_{30}$ film, respectively. These results supported the idea that the mechanical properties of the polymer film could be tuned easily by incorporating high-strength materials like GO within polymer architecture.

7.3 The Application of the LbL-Assembled Layer Architectures in Biomedicine

The simplicity, versatility, and nanoscale control that the LbL assembly provides has made it a promising candidate in biomedical fields like biosensing, drug delivery, cellular engineering, tissue engineering, and regeneration medicine [42–44]. There are hundreds of papers in these fields published each year since its first introduction.

7.3.1 Biosensing

From measuring body activity to critical health statistics, biosensing technologies are promoting progresses in healthcare and wellness. Biosensors are

analytical devices that incorporate biological sensing elements, for which the immobilization of the biomolecules is a crucial step due to their easy denaturation by changes in environment or surrounding conditions [45]. Thus, proper orientation of the biological sensing elements with negligible conformational changes plays an important role in fabricating a sensitive and selective biosensor. At this point, the LbL assembly has proved to be an excellent choice. Various LbL-assembled architectures have been studied to incorporate antibodies, enzymes, proteins, and DNA in the organized films for the design of biosensors [46–49].

We have co-immobilized glucose oxidase (GOx) and graphene to fabricate a glucose biosensor [46], as shown in Figure 7.5. This work demonstrated the capability of graphene as the spacer to form electrochemically functionalized inorganic and organic hybrid nanoarchitecture. It was demonstrated that the developed glucose biosensor allowed rapid and sensitive determination of

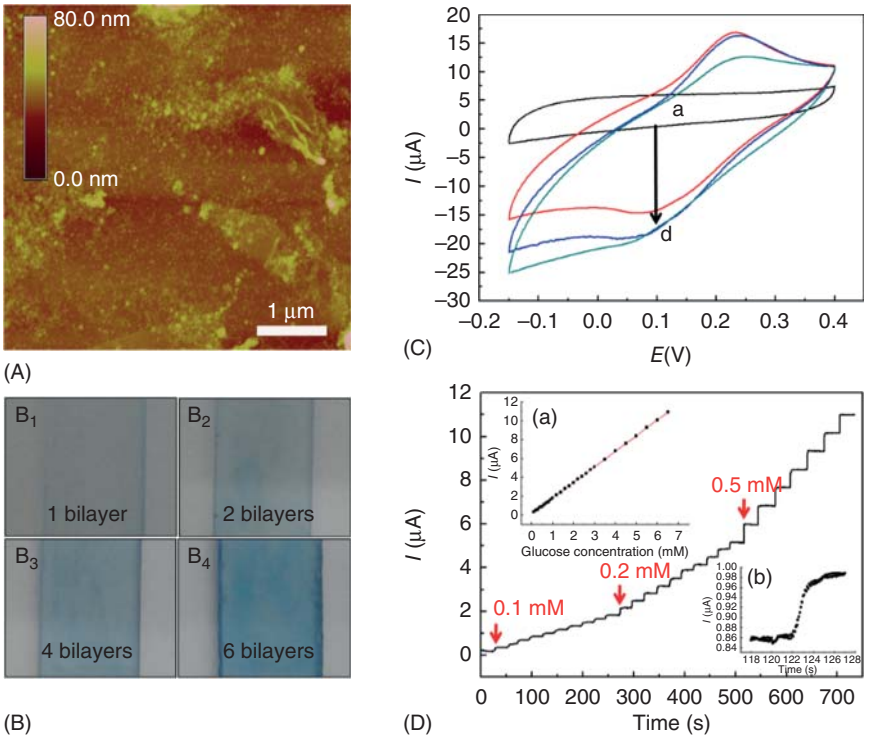

Figure 7.5 (A) Atomic force microscopy (AFM) image of (graphene/PDDA-PB)$_1$ ultrathin film assembled on silicon wafer. (B) Photographs of (graphene/PDDAPB)n films assembled on quartz slides with increasing bilayer numbers (from one bilayer to six bilayers). (C) Cyclic voltammograms of the bare glass carbon electrode (GCE) in the presence of glucose solution (5.0 mM) (a) and the (graphene/PDDA-PB/GOx/PDDA-PB)$_3$ film electrode in the absence (b) and presence of 2.0 mM (c), and 5.0 mM glucose solution (d) in phosphate buffer saline (PBS) (pH 7.4) containing 0.1 M KCl at 50 mV s^{-1}. (D) Current–time amperometric response of (graphene/PDDA-PB/GOx/PDDA-PB)$_3$ film electrode with successive addition of glucose into stirring PBS of pH 7.4, containing 0.1 M KCl. Inset (a) is the calibration curve. Inset (b) shows the steady-state current response time of the modified electrode to 0.1 mM glucose. Applied potential: 0.2 V. (Yan *et al*. 2015 [46]. Reproduced with permission of Springer.)

glucose. The amperometric response exhibited a linear correlation to glucose concentration over a range from 0.1 to 6.5 mM and the response time and detection limit were determined to be 2 s and 6 μM, respectively.

7.3.2 Drug Delivery

In biomedical applications, the field of drug/active agent delivery is undoubtedly the most potential application of the LbL-assembled systems [50]. Its versatility is the biggest advantage of this technique, by which biocompatible and nanostructured materials for drug delivery can be easily embedded into the films. Several groups have reported the delivery of growth factors via the layered architectures. For instance, Picart and coworkers have performed several works focusing on the assembly of polymer multilayer loaded with bone morphogenetic proteins (BMPs) [51–53]. Figure 7.6 summarizes the major differences between matrix-bound and soluble delivery of BMP-2 to the cell. They have shown the osteoinductive character of the BMP-loaded multilayers in a rat ectopic model. Moreover, by studying the secondary structure of rhBMP-2, they also showed that the growth factor retained its bioactivity when the films were stored in the hydrated and, to a lesser extent, in the dry state [54].

7.3.3 Cellular and Tissue Engineering

The LbL assembly technique makes it possible to impart cell adhesive properties and biocompatibility to an existing substrate while still maintaining the desired characteristics of the substrate, which is useful in cell therapy, cellular and tissue engineering, and regenerative medicine [55–57]. Their usage usually includes the effect on either promoting or preventing cell adhesion and, more importantly, on directing or maintaining cellular phenotype [58, 59].

Simultaneously, the ease of controlling the structure of LbL films provides the possibility of manipulating adhesion, proliferation, differentiation, and even functions of the attached cells on the films at the molecular levels. Some kinds of the LbL multilayer have been used to modulate protein adsorption, or to regulate the inflammatory response [60, 61]. For example, a kind of composite layer composed of GO and poly-L-lysine (PLL) has been investigated for the differentiation of mesenchymal stem cells (MSCs) [62]. The results demonstrated that the GO/PLL composite films could not only support the growth of MSCs with a high proliferation rate but could also accelerate the osteogenic differentiation of MSCs, showing strong alkaline phosphatase (ALP) activity and gene expression.

In addition, the layered architectures are attractive and have potential in the field of cellular and tissue engineering also due to the ability to render implantable material surfaces more biomimetic since they have the potential to mimic the extracellular matrix (ECM) *in vivo*. A good example is the collagen-based layered architectures, which have been constructed with chitosan, alginate, and other biopolymers via the LbL assembly, respectively [63–66].

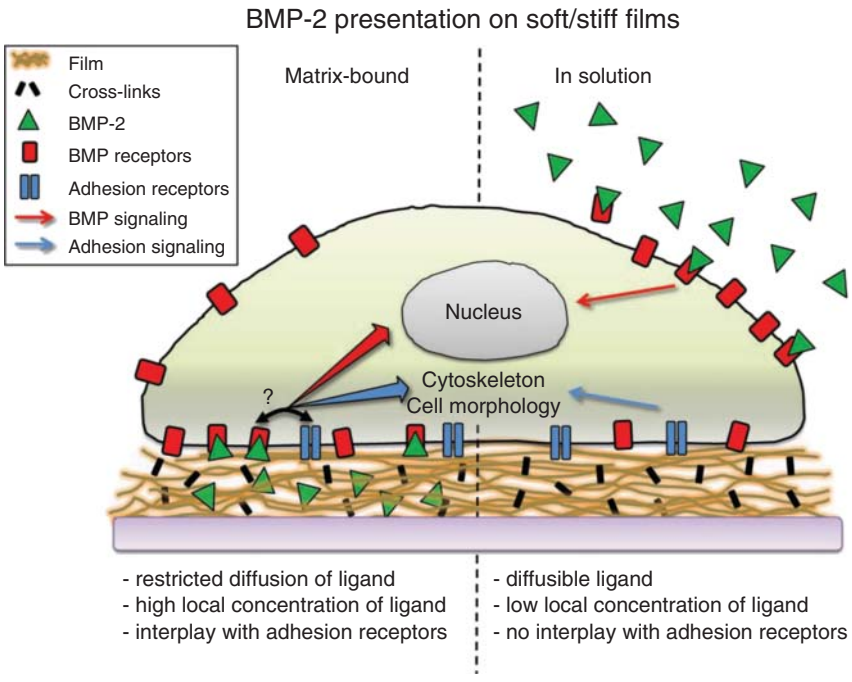

Figure 7.6 Schematic representation of the difference of BMP-2 presentation on films and in solution to the cell. When BMP-2 is bound to the film (left part of the scheme), it is spatially confined and its diffusion is restricted. In addition, the occupancy rate of BMP-2 receptors is enhanced with a possible formation of homo- and heterodimeric receptor complexes and ligand/receptor binding is not limited by diffusion (a high number of free ligands is available in the proximity of the receptors). Furthermore, due to the close proximity of growth factor receptors and adhesion receptors, a cross talk between these two types of receptor is possible. Thus, cross talks between BMP-2 signaling and adhesion signaling, which can induce cytoskeleton remodeling, might explain the striking effects observed for cells plated on soft films with bBMP-2. Such a cooperative effect cannot be observed when BMP-2 is presented in solution (right part of the scheme), that is, BMP-2 can freely diffuse in 3D and has a low availability due to the diffusion-limited reaction between receptors and ligands. Furthermore, in this case, BMP-2 receptors are diffusing at the plasma membrane and are not in the vicinity of adhesion receptors. (Crouzier *et al*. 2011 [53]. Reproduced with permission of John Wiley & Sons.)

7.4 Summary and Outlook

In summary, the LbL assembly technique, one of the nanoarchitectonic approaches, represents a simple, efficient, and highly versatile method which allows for the integration of a large variety of materials into layered architectures with tunable structures, compositions, properties, and functions. Since its first introduction, it has been showing its wide application in energy storage, solar cells, catalysis, gas separation, and especially in the biomedical field.

Although remarkable developments have been achieved in the previous studies, undoubtedly, many stimulating challenges and opportunities still await us to explore the potential of the LbL architectures in the coming future, for instance, the commercially based merits and industrial scalability.

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